

## WHAT IS CLAIMED IS:

1. A host cell producing a high mannose recombinant protein, comprising a polynucleotide encoding the recombinant protein and a signal for causing the recombinant protein to be produced as a high mannose protein.
2. The cell of claim 1, wherein said polynucleotide comprises a first nucleic acid sequence encoding said protein of interest operably linked to a second nucleic acid sequence encoding a signal peptide.
3. The cell of claim 2, wherein said signal peptide comprises an ER (endoplasmic reticulum) targeting signal peptide.
4. The cell of claim 3, wherein said polynucleotide further comprises a third nucleic acid sequence for encoding a vacuolar targeting signal peptide.
5. The cell of claim 1, wherein said signal causes the recombinant protein to be targeted to the ER.
6. The cell of claim 5, wherein said signal comprises a signal peptide for causing the recombinant protein to be targeted to the ER.
7. The cell of claim 6, wherein said polynucleotide comprises a nucleic acid segment for encoding said signal peptide.
8. The cell of claim 1 or any of claims 3-7, wherein said signal causes the recombinant protein to by-pass the Golgi.
9. The cell of claim 8, wherein said signal comprises a signal peptide for causing the recombinant protein to not be targeted to the Golgi.

10. The cell of claim 9, wherein said polynucleotide comprises a nucleic acid segment for encoding said signal peptide.
11. The host cell according to claim 1, wherein said host cell is any one of a eukaryotic and a prokaryotic cell.
12. The host cell according to claim 11, wherein said prokaryotic cell is a bacterial cell, preferably an *Agrobacterium tumefaciens* cell.
13. The host cell according to claim 12, wherein said eukaryotic cell is a plant cell.
14. The host cell according to claim 13, wherein said plant cell is a plant root cell selected from the group consisting of *Agrobacterium rhizogenes* transformed root cell, celery cell, ginger cell, horseradish cell and carrot cell.
15. The host cell according to claim 14, wherein said plant root cell is a carrot cell.
16. The host cell according to claim 15, wherein said recombinant polynucleotide comprises a first nucleic acid sequence encoding said protein of interest that is in operable link with a second nucleic acid sequence encoding a vacuolar targeting signal peptide derived from the basic tobacco chitinase A gene, which vacuolar signal peptide has the amino acid sequence as denoted by SEQ ID NO: 2, wherein said first nucleic acid sequence is optionally further operably linked to a third nucleic acid sequence encoding an ER (endoplasmic reticulum) targeting signal peptide as denoted by SEQ ID NO: 1.
17. The host cell according to claim 16, wherein said recombinant polynucleotide further comprises a promoter that is functional in plant cells, wherein said promoter is operably linked to said recombinant molecule.

18. The host cell according to claim 17, wherein said recombinant polynucleotide further comprises a terminator that is functional in plant cells, wherein said terminator is operably linked to said recombinant molecule.

19. The host cell according to claim 18, wherein said recombinant polynucleotide optionally further comprises additional control, promoting and regulatory elements and/or selectable markers, wherein said regulatory elements are operably linked to said recombinant molecule.

20. The host cell according to claim 19, wherein said high mannose protein is a high mannose glycoprotein having glycosylation with at least one exposed mannose residue.

21. The host cell according to claim 20, wherein said high mannose protein is a biologically active high mannose lysosomal enzyme selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase,  $\alpha$ -N-acetylgalactosaminidase, acid lipase,  $\alpha$ -galactosidase, glucocerebrosidase,  $\alpha$ -L-iduronidase, iduronate sulfatase,  $\alpha$ -mannosidase and sialidase

22. The host cell according to claim 21, wherein said lysosomal enzyme is human glucocerebrosidase (GCD).

23. The host cell according to claim 22, wherein said GCD comprises the amino acid sequence substantially as denoted by SEQ ID NO: 8, encoded by the nucleic acid sequence as denoted by SEQ ID NO: 7.

24. The host cell according to claim 23, wherein said cell is transformed or transfected with a recombinant polynucleotide or with an expression vector comprising said molecule, which recombinant polynucleotide further comprises an  $^{35}\text{S}$  promoter from Cauliflower Mosaic Virus, an octopine synthase terminator of

*Agrobacterium tumefaciens*, and the regulatory element is the TMV (Tobacco Mosaic Virus) omega translational enhancer element, and having the nucleic acid sequence substantially as denoted by SEQ ID NO: 13 encoding GCD having the amino acid sequence substantially as denoted by SEQ ID NO: 14.

25. A recombinant high mannose protein produced by the host cell according to any one of claims 1 to 24.

26. The recombinant high mannose protein according to claim 25, wherein said high mannose protein is a biologically active high mannose lysosomal enzyme selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase,  $\alpha$ -N-acetylgalactosaminidase, acid lipase,  $\alpha$ -galactosidase, glucocerebrosidase,  $\alpha$ -L-iduronidase, iduronate sulfatase,  $\alpha$ -mannosidase and sialidase.

27. The recombinant protein according to claim 26, wherein said lysosomal enzyme is human glucocerebrosidase (GCD).

28. A recombinant biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue.

29. A recombinant protein, comprising a first portion having signal peptide activity and a second portion having lysosomal enzyme activity, said first portion causing said second portion to be processed in a plant cell with at least one oligosaccharide chain comprising an exposed mannose residue.

30. The protein of claim 29, wherein said lysosomal enzyme comprises a protein for the treatment or prevention of Gaucher's disease.

31. The protein of claims 29 or 30, wherein said protein comprises hGCD.

32. The protein of any of claims 29-31, wherein said first portion comprises a plant cell ER targeting signal peptide.

33. The recombinant lysosomal enzyme according to claim 29, wherein said recombinant enzyme can bind to a mannose receptor on a target cell in a target site within a subject suffering from a lysosomal storage disease.

34. The recombinant lysosomal enzyme according to claim 33, wherein said recombinant lysosomal enzyme has increased affinity for said target cell, in comparison with the corresponding affinity of a naturally occurring lysosomal enzyme for said target cell.

35. The recombinant lysosomal enzyme according to claim 34, wherein said recombinant lysosomal enzyme is selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase,  $\alpha$ -N-acetylgalactosaminidase, acid lipase,  $\alpha$ -galactosidase, glucocerebrosidase,  $\alpha$ -L-iduronidase, iduronate sulfatase,  $\alpha$ -mannosidase or sialidase.

36. The recombinant lysosomal enzyme according to claim 35, wherein said recombinant lysosomal enzyme is glucocerebrosidase (GCD).

37. The recombinant lysosomal enzyme according to claim 36, wherein said target cell at the target site is a Kupffer cell in the liver of said subject.

38. A recombinant high mannose protein, produced in plant cell culture.

39. The recombinant high mannose protein of claim 38, comprising a plant signal peptide for targeting a protein to the ER.

40. The protein of claim 39, wherein said plant signal peptide comprises a peptide for targeting said protein to the ER in a root plant cell culture.

41. The protein of claim 40, wherein said root plant cell culture comprises carrot cells.
42. A recombinant high mannose hGCD protein, produced in plant cell culture.
43. Use of a plant cell culture for producing a high mannose protein.
44. A method of producing a high mannose protein comprising:  
preparing a culture of recombinant host cells transformed or transfected with a recombinant polynucleotide encoding for a recombinant protein;  
culturing said host cell culture under conditions permitting the expression of said protein, wherein said host cells produce said protein in a highly mannosylated form.
45. The method of claim 44, wherein said host cell culture is cultured in suspension.
46. The method of claim 45, further comprising:  
purifying said protein.
47. The method according to claim 45, wherein said host cell is as defined by any one of claims 1 to 15.
48. The method according to any one of claims 46 or 47, wherein said high mannose protein is a biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue.
49. The method according to claim 48, wherein said recombinant enzyme binds to a mannose receptor on a target cell in a target site.

50. The method according to claim 49, wherein said recombinant enzyme has increased affinity for said target cell, in comparison with the corresponding affinity of a naturally occurring lysosomal enzyme to said target cell.

51. The method according to claim 50, wherein said lysosomal enzyme is selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase,  $\alpha$ -N-acetylgalactosaminidase, acid lipase,  $\alpha$ -galactosidase, glucocerebrosidase,  $\alpha$ -L-iduronidase, iduronate sulfatase,  $\alpha$ -mannosidase and sialidase.

52. The method molecule according to claim 51, wherein said lysosomal enzyme is glucocerebrosidase (GCD).

53. The method according to claim 52, wherein said target cell at the target site is Kupffer cell in the liver of said subject.

54. The method according to claim 53, wherein said host cell is a plant root cell selected from the group consisting of *Agrobacterium rhizogenes* transformed root cell, celery cell, ginger cell, horseradish cell and carrot cell.

55. The method according to claim 54, wherein said plant root cell is a carrot cell.

56. The method according to claim 55, wherein said transformed host carrot cells are grown in suspension.

57. A method for treating a subject having lysosomal storage disease using exogenous recombinant lysosomal enzyme, comprising:

- (a) providing a recombinant biologically active form of lysosomal enzyme purified from transformed plant root cells, and capable of efficiently targeting cells abnormally deficient in said lysosomal enzyme,

wherein the recombinant biologically active enzyme has exposed terminal mannose residues on appended oligosaccharides; and

(b) administering a therapeutically effective amount of said recombinant biologically active lysosomal enzyme to said subject.

58. The method according to claim 57, wherein said host cell is as defined by any of claims 1-24.

59. The method according to claim 58, wherein said host cell is a carrot cell.

60. The method according to claim 59, wherein said lysosomal enzyme is a high mannose enzyme comprising at least one oligosaccharide chain having an exposed mannose residue.

61. The method according to claim 60, wherein said recombinant enzyme can bind to a mannose receptor on a target cell in a target site within a subject.

62. The method according to claim 61, wherein said recombinant lysosomal enzyme has increased affinity for said target cell, in comparison with the corresponding affinity of a naturally occurring lysosomal enzyme to said target cell.

63. The method according to claim 62, wherein said lysosomal enzyme is selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase,  $\alpha$ -N-acetylgalactosaminidase, acid lipase,  $\alpha$ -galactosidase, glucocerebrosidase,  $\alpha$ -L-iduronidase, iduronate sulfatase,  $\alpha$ -mannosidase or sialidase.

64. The method according to claim 63, wherein said lysosomal enzyme is glucocerebrosidase (GCD).



65. The method according to claim 64, wherein said lysosomal storage disease is Gaucher's disease.

66. The method according to claim 65, wherein said target cell at the target site is a Kupffer cell in the liver of said subject.

67. A pharmaceutical composition for the treatment of a lysosomal storage disease comprising as an active ingredient a recombinant biologically active high mannose lysosomal enzyme as defined by any one of claims 25-42, which composition optionally further comprises pharmaceutically acceptable dilluent, carrier or excipient.

68. The composition according to claim 67, wherein said lysosomal storage disease is Gaucher's disease.

69. The composition according to claim 68, wherein said recombinant lysosomal enzyme is a biologically active high mannose human glucocerebrosidase (GCD).

70. Use of a recombinant biologically active high mannose lysosomal enzyme as defined by any one of claims 25-42, in the manufacture of a medicament for the treatment or prevention of a lysosomal storage disease.

71. The use according to claim 70, wherein said disease is Gaucher's disease.

72. The use according to claim 71, wherein said biologically active lysosomal enzyme is a biologically active high mannose human glucocerebrosidase (GCD).